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Bacteria Isolated from PUS Samples of the Bacteriological Profile and Anti-Biogram in a Secondary Care Unit

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ABSTRACT

The human pus sample is the most important obtained from the culturing Microbiology Laboratory and the antimicrobial sensitivity research. A total of 615 pus samples were processed for aerobic cultivation and sensitivity research and antibiotic susceptibility testing in a variety of IPD or OPDs of Secondary Care Hospital in Namakkal District. The analysis of 615 pus samples indicated that 67.31% of culture positive (Male:Female Ratio=1.30:1.00) was highest contributors to Surgical Wards (58.94%). The most frequent organism, followed by Enterococcus sp, is S.aureus (25.30 percent) (18.80 percent). Gram positive bacteria exhibited sensitivity to Meropenem (100%), Amikacin (90.7%), Piperazylin (67.8%) and Gram-negatives were extremely sensitive to Meropenem (98.2%) and Doxycycline Antibiotic sensitivity profile (69.0 percent). There was a significant incidence of MRSA (50 percent). Bacterial DNA was isolated and 16S rRNA sequence was sequenced to distinguish and define taxonomic classification of the very highly antibiotic-resistant bacteria. The isolates of Pseudomonas aeruginosa have been confirmed to be resistant to amikacin, chloramphenicol, penicillin-G, tetracycline and vancomycin by 16s rRNA sequencing. In view of the increase in antibiotic resistance the antimicrobial susceptibility of bacterial isolates from pus samples has been evaluated to choose suitable medications for prevention and treatment of illnesses.

KEYWORDS: *Pyogenic infection, Pus, Microbial profile, Susceptibility, S. aureus*

I. INTRODUCTION

The production of pus/abscesses results from inflammatory responses to tissue infection, including skin, ear, tooth, brain, liver, abdomen, uterus, etc. Pyogenic bacteria, including Staphylococcus aureus, S. epidermidis, S.pyogenes, Escherchia coli, Streptococcus pneumonia, Clebsiella pneumonia, Pseudiomonas aeruginosa and Mycobacterium TB, are important contributors of abscess/pus development. The immune system releases antibodies into the diseased region in order to fight infections (Abbas AK, Lichtman AH 2014). Staphylococcus aureus was the dominating organism in the pus, followed by Streptococcus species (Lee et al. 2018). This infection causes significant disease, extended

hospitalisation and a huge economic impact (Kobayashi and DeLeo 2018). In addition, antibiotic resistance among these pyogenic bacteria leads to poor therapy and illness management. The growing frequency of drug resistance, including S. aureus methicillin-resistant strains and the development of biofilms, results in the treatment of these multi-drogue-resistant bacteria and community spread. The medication susceptibility patterns/antibiograms of pus/wound swab isolated bacterial strains may be utilised for understanding and treating sensitive or resistant pyogenic bacteria.

Several investigations have shown various bacterial samples of liver, brain, skin, and abdomen isolated from pus and abscesses (Dinda et al. 2013; Reyna-Fabián et al. 2016; Fan et al. 2017; Belbase et al. 2017; Sapkota et al. 2019). The bacterial groupings' antibiotic patterns reveal a growing tendency towards antibiotic resistance and this pattern is related to several risk factors. Sporadic clinical reports can assist physicians and policymakers forecast and control the expansion of pyogenic antibiotic resistance bacteria. A research was thus undertaken at a Namakkal District secondary-care hospital in India to examine the shifting trends in antibiotic resistance in different pus isolates. Different predictors and risk variables for isolated pyogenic bacteria antibiograms were understood.

II. Materials and Methods

A. Sample collection

This is a prospective research, in which a total of 615 pus samples were collected from diverse sources in the hospitals in the Namakkal district at the hospital's Inpatient Departments (IPDs) and Outpatient Departments (OPDs). Isolation and sensitivity patterns against commercially available antibiotics of aerobic microbial culture have been investigated in order to predict the antibiogram of the isolated strains.

B. Isolation of pus associated bacterial pathogens

Sterile disposable cotton swabs and syringes were taken to collect Pus samples from patients, and then transported and processed promptly under laboratory settings. Blood agar (BA), MacConkey agar (MA) and Nutrient agar (NA) media inocular samples were used. The cultivation plates were incubated at 37°C for 24 hours. Following isolation, traditional identification procedures such as catalase, coagulase, indole, methyl-red, Voges-Proscauer, citrate, urease, phenyl pyruvic acid and oxidase were detected for pathogenic strains (Parajuli et al. 2014).

C. Antibiotic sensitivity test

The antibiotic sensitivity tests (Raza and al. 2013) were conducted on Muller Hinton agar, explaining the sensitivity pattern of pus-isolated, classified as sensitive or resistant pathogenic bacterial strains, in accordance with the CLSI protocol (Clinical and Laboratory Standard Institute, 2012). MRSA isolate drug sensitivity was detected using Cefoxitin discs (30 µg) and S. aureus ATCC 25923 and E. coli ATCC 25922 as a controller (Chakraborty et al., 2011). Amikacine (30 µg), chloramphenicol (25 µg), penicillin-G (2U/Disc), tetracycline (30 µg), and vancomycin (30 µg), meropenem (10 mcg), phosphate (30 mcg), gentamycin (5 mcg) and cefopérazone (75 mcg), ciprofloxacin (5 mcg), norfloxacin (10 mcg), nitrofuratoin (300 mcg), azithromycin (15 mcg), Ofloxacin(5 mcg).

D. Genomic DNA isolation and PCR

The antibiotic resistant isolates of Pseudomonas sp. were homogenised using the Lysing Matrix-A tool FastPrep®-24 (MP Biomedicals, USA). The DNA was extracted in accordance with manufacturer's instructions using a high pure PCR template preparation kit (Roche, Germany). The universal primers were produced (Raja et al. 2017) and the cycles were determined to be denatured by 2 minutes at 95 ° C, 1 minutes by 35 cycles at 95 ° C, 45 s by 52 ° and 5 minutes by the end extension at 72 ° C. All additional steps as per our past work have been followed (Raja et al. 2017).

E. Phylogenetic analysis of Antibiotic resistant Pseudomonas sp. isolates

The NCBI database has recovered closely related sequences and phylogenetic analysis is carried out with the Maximum Likelihood (ML) technique. The tree with the highest log probability is displayed (-2494.36). The original heuristic search tree(s) were automatically produced by utilising Neighbor-Join and BioNJ algorithms for a matrix of pair wise distances evaluated using the Tamura-Nei model and the topology with a higher value in log probability. Five nucleotide sequences were involved in this investigation. In the final dataset there were a total of 1581 locations. In MEGA X evolutionary analyses were performed (Kumar et al. 2018).

III. Results and Discussion

Of the 615 pus samples taken for aerobic culture and antibiotic sensitivity at Namakkal Hospital IPDs and IPDs, 67.3% (414) of the samples were positive, 32.68%(201) of which had no bacterial growth (Fig. 1A). 234 (56.6%) of the 414 samples were male and 180 (43.4%), female (Fig. 1B) were male: female risk ratio 1,30:1.00. The departmentally positive distribution of the pus samples for aerobic cultures shows a major contributor of the surgical department (58.94%), followed by an OPD (14.01%), the general medicine department, including the eye, paediatrics (12.32%), the obstetrics and gynaecology departments (OG: 11.11%, etc.) and the Trauma Care Department (3.62%). (Fig. 1C). Based on biochemical assays, pyogene bacteria isolated from the pus samples were identified and isolated.

Most of the pus specimens reveal the presence of Gram positive bacteria Staphylococcus Sp. (25.12%) followed by other insulates such as Enterococcus (18.80%), Proteus sp. (14.70%), E. coli (13.73%) and Pseudomonas Sp (8.43 percent). Negative coagulase Staphylococcus (CONS: 4.11%) also contributed to pyogenic bacteria collection. In our investigation, the predominant cause for pyogenic abrasion corroborated by the previous study was a Gram-negative bacterium (Zubair et al. 2011).

Antibiotic resistance is a major concern and a continual review is essential in order to detect the susceptibility of these infections and to pick the appropriate prophylactic and treatment regimens. In contrast to other antibiotics examined in this study, meropenem and amikacin have been found to be effective in treating Gram positive and negative bacteria. Similar investigations have been carried out in a tertiary hospital to investigate the antibiotic sensitivity to bacteria isolated from different pus cultures (Tiwari and Kaur 2010).

S. aureus is the most prevalent gram-positive isolate in our research (25%) as demonstrated in prior studies (Lee et al. 2009; Tiwari and Kaur 2010). However, subsequent research (Kshetry et al. 2016) (37.6%), (Sanjana et al. 2010) (39.6%), (Dibah et al. 2014) (46.3%) and (Tiwari et al. 2009) have shown greater rates (69.1 percent). In some studies the variation in MRSA prevalence may be attributed to inappropriate antibiotic in the pyogenic bacteria selection. Meropenem (100 per cent) and Amikacin (90.7 per cent) were also sensitive to Staphylococcus sp. (Samra et al. 2005) and reported 100 per cent sensitivity. Gram-negative bacteria have a high sensitivity antibiotic profile for Meropenem (98.2 percent) followed by Doxycycline (69.0 percent) and Amikacin (65.7 percent) (Fig. 2A-B).

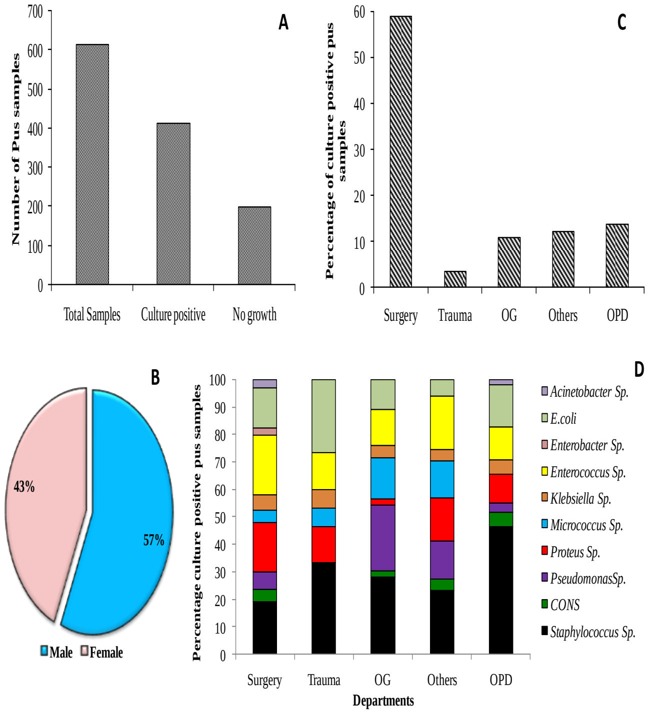

Figure 1. (a) Cultural positive case distribution, (b) the gender-sensitive distribution of pathogen positive sample patients, (c) the wise distribution of pus samples and the bacterial pathogens' wise distribution department

Pseudomonas isolates (PS1-4) are found to be Amikacin resistant (30 µg), Penicillin-G resistant (25 µg), Tetracycline resistant (30 µg) and Vanicomycin resistant (30 µg) (Fig 3). The four resistant isolates were sequenced using 16S rRNA followed by development of a phylogenetic tree. Maximum Likelihood Method and Tamura-Nei Model have led to evolutionary history (Tamura and Nei 1993). The four isolates were identical to the aeruginosa Pseudomonas. About 46 Pseudomonas isolates were recovered mostly of Pus, Wound Swab, Sputum and Tracheal Aspirates with 63.04% P. aeruginosa isolates, Ceftazidime resistant 65.21%, Ceftriaxone and Cefotaxime 56.52%, and Piperacillin resistant 56.52%, respectively (Pokharel et al., 2019). Similarly, the majority (n=82) of 200 P. aeruginosa isolates at the tertiary health centre, Faisalabad, was recovered from β-lactam resistant pus samples, including carbapenems, followed by 95% by levofloxacin and 67% by doxyl cycline (Qureshi et al., 2018).

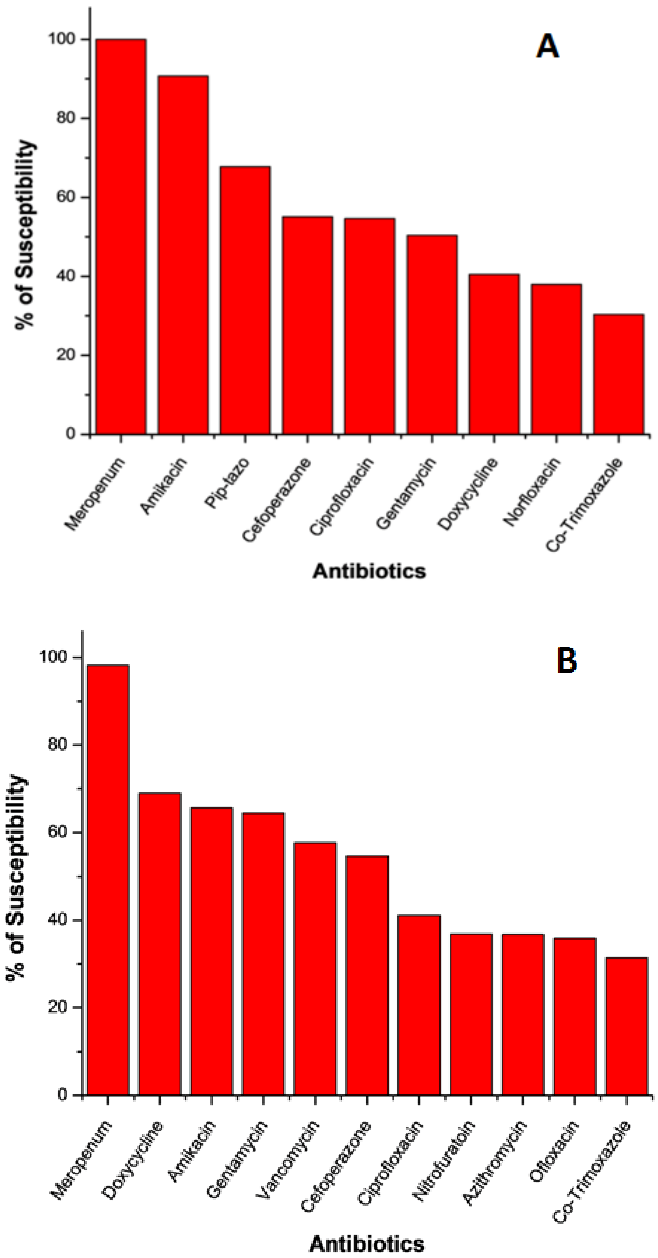

Figure 2.Antibiotic susceptibility pattern of (A) Gram positive and (B) Gram negative Bacteria isolated from pus samples

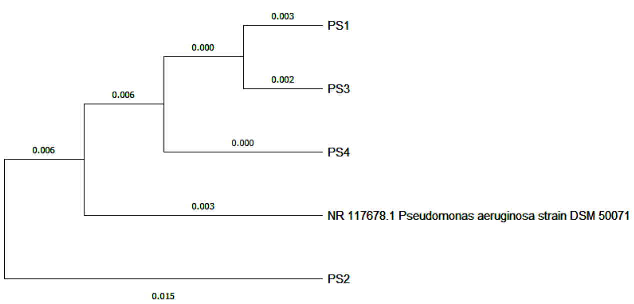

Figure 3. Antimicrobial resistance of P. aeruginosa isolates (PS1-4) against Amikacin (Ak-30 µg), Chloramphenicol (C-25 µg), Penicillin- G (P-2U/Disc), Tetracycline (TE-30 µg) and Vancomycin (VA-30 µg)

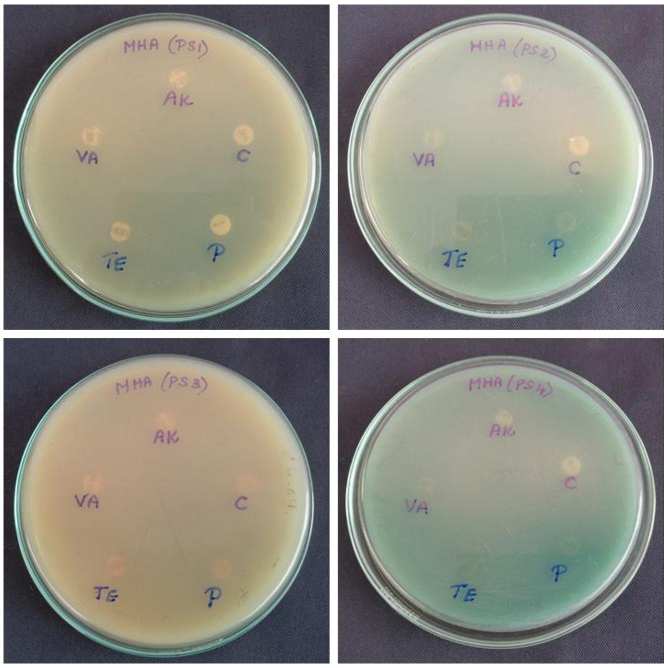

Figure 4. Evolutionary analysis of antibiotic resistant Pseudomonas sp. by Maximum Likelihood method

IV. Conclusion

The current paper discusses a superior grasp of the causative microbial pathogens found in the wound/pus infections and their sensitivity and resistance profiles to existing medications. In most cases, pus infections have been linked with Staphylococcus, Klebsiella, Escherchia, Pseudomonas aeruginosa, Enterococcus proteus, Micrococcus etc. Antibiotic resistance is a major concern, with high antibiotic resistance results also showing in isolated bacteria. Meropenem and amikacin were more likely to be successful for treating gramme, as opposed to the other antibiotics evaluated in this study, among the antibiotics examined. Four Pseudomonas (PS1-4) isolates were discovered to be amikacin, chloramphenicol, penicillin-G, tetracycline and vancomycin resistant. Pseudomonas aeruginosa verified by 16S rRNA sequence followed by phylogenetic tree building were the four resistant isolates. In order to assess infections susceptibility and pick suitable regimes for prevention and treatment, an ongoing evaluation is needed.

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